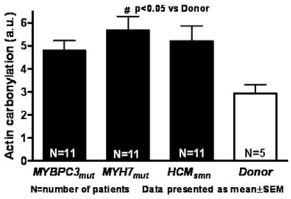
and chemiluminescence using appropriate antibodies. Actin-carbonylation was higher in HCM patients compared to donors. It was highest in MYH7mut(Figure).

Conclusion: Increased actin-carbonylation could partially underlie reduced force development in human HCM. Increased oxidative stress may contribute to impaired contractile function during disease development in sarcomere mutation-positive and mutation-negative HCM patients.



Microtubules, Their Motors, and Associated Proteins II

3925-Pos Board B653

Structural Kinetics of the Mitotic Kinesin Eg5

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Members of the kinesin superfamily of molecular motors differ in several key structural domains, which probably allow these molecular motors to serve the different physiologies required of them. One of the most variable of these is a stem-loop motif referred to as L5. This loop is longest in the mitotic kinesin Eg5, and previous structural studies have shown that it can assume different conformations in different nucleotide states. However enzymatic domains often consist of a mixture of conformations whose distribution shifts in response to substrate binding or product release, and this information is not available from the "static" images that structural studies provide. We have addressed this issue in the case of Eg5 by attaching a fluorescent probe to L5 and examining its fluorescence, using both steady state and time-resolved methods. This reveals that L5 assumes an equilibrium mixture of three orientations that differ in their local environment and segmental mobility. Combining these studies with transient state kinetics demonstrates that there is a major shift in this distribution during transitions that interconvert weak and strong microtubule binding states. Finally, in conjunction with cryoEM reconstructions of Eg5:microtubule complexes, these fluorescence studies suggest a model in which L5 regulates both nucleotide and microtubule binding through a set of reversible interactions with helix α3. We propose that these features facilitate the production of sustained opposing force by Eg5, which underlies its role in supporting formation of a bipolar spindle in mitosis.

3926-Pos Board B654

Structural Basis for the Assembly of Kinesin-5 into Bipolar Anti-Parallel Tetramers

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Cell division is driven by organized mitotic spindles and requires diverse groups of molecular microtubule-based kinesin motor proteins. Kinesin-5 motors form a unique and conserved class of tetrameric bipolar motor proteins, consisting of dual motor domain sets oriented at opposite ends of a central rod-like structure. The bipolar organization is critical for kinesin-5 to crosslink and slide adjacent anti-parallel microtubules in opposite directions required for spindle elongation in anaphase. Here we present the atomic structure of Drosphila kinesin-5, KLF61F, bipolar assembly (BASS) domain, which explains how four molecules assemble to form a bipolar functional motor. Instead of the predicted dual traversing parallel coiled-coils, the BASS structure reveals a novel 26nm long antiparallel 4-helical bundle filament, where the basic assembly unit is an anti-parallel coiled-coil that originate from opposite ends of the bipolar structure, and folds onto a second unit in an anti-parallel manner. A striated pattern of hydrophobic and hydrophilic pockets precisely oriented monomers within the BASS tetramer to form this bipolar arrangement. Strikingly, the BASS N-termini undergo a helical exchange from anti-parallel bundle in the center to form homo-dimeric parallel helical coiled-coils at the poles. These parallel BASS N-termini are 100 degrees rotated compared to N-termini emerging from the opposite pole, providing a physical explanation for the known kinesin-5 preference for sliding anti-parallel versus parallel microtubules. We have generated a structural model for the kinesin-5 tetramer using the BASS structures and all electron microscopy that explains key features of the kinesin-5-driven sliding filament mechanism.

3927-Pos Board B655

Kinesin-5 Motility is Regulated by the Residue Chemistry of Loop-5

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Kinesin-5 drives separation of microtubules and organizes the mitotic spindle via its motor domains. More than one component within Kinesin-5 has been implicated in its motile ability; these are Loop-5, the necklinker and the cover neck. In all studies, measured changes in structure, or their implied dynamic motion, were deemed key to the role of these components. Unaddressed is whether these protein components chemically direct mechanical output. Herein, we show that specific side-chain chemistry of Loop-5 must be conserved for productive Kinesin-5 motility. Six substitutions of Loop-5 residues were created in the Eg5 dimer by mutagenesis, and their microtubule-gliding velocities were measured. Mutant Eg5 gliding velocities ranged from nearly equivalent to wildtype to a complete loss of motility. There were key differences between the mutations in the N- versus C-terminus of Loop-5. Substitutions near the N-terminus retained the ability to glide microtubules; alteration of gliding velocity paralleled changes in microtubule-stimulated catalytic rates. In contrast, nonconservative substitutions near the C-terminus of Loop-5 bound microtubules in rigor despite having robust ATPase activity. Collectively, these results suggest the integrity of the active site remains intact and communication between the active site and microtubule site is not compromised. Mechanical output is challenged, however. Therefore, we conclude that side-chain interactions of these C-terminal residues with the surrounding protein matrix are required for the terminal step in Eg5 mechanotransduction. Given our kinetic data, we speculate that aberrant interactions may result in changes in force and/or coordination of the two motor domains in the dimer, rendering Eg5 incapable of motility. This work is funded by the support of the National Institutes of Health (R01 GM097350 S.K.;P20GM103424 and 5G12RR026260 T.H.) and the LSU School of Graduate Studies (R.B.).

3928-Pos Board B656

A Chimeric Kinesin-5 Motor Tracks Plus-Ends of Microtubules

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Kinesin-5 is necessary for bipolar spindle formation during mitosis. Besides its unique homotetrameric configuration, determined by the coiled-coil domain, the kinesin-5 motor domain also possesses specific properties suitable for its spindle organizing function. To study the walking properties of functional kinesin-5 dimers, we fused the kinesin-5 head and neck linker domain to the coiled-coil rod of kinesin-1. We report that a chimeric kinesin-5 head/kinesin-1 rod construct with 14 aa neck linker tracks plus-ends of both growing and static (taxol-stabilized) microtubules with a mean residence time of roughly 7 seconds. The same construct having a 18 aa neck linker, as found in wild-type kinesin-5, also remained bound to the plus-end of static microtubules, but labeling at growing microtubule plus-ends was not observed. These phenomena explain previous reported end-to-end tethering of microtubules following antiparallel sliding by full length kinesin-5. This end-labeling presumably occurs because the motor walks to the end of the microtubule and is unable to take a subsequent step. We hypothesize that kinesin-5 remains bound at the plus-ends of microtubule by a single head in the ATP state and that hydrolysis and dissociation of this head requires binding of the tethered head to the next binding site on the microtubule. Computational modeling based on this hypothesis predicts that motor accumulation at the plus-ends of growing microtubules relies on motor processivity, consistent with experimental observations. Our studies reveal a property intrinsic to the kinesin-5 head domain that is distinct from transport motors such as kinesin-1 and kinesin-2.

3929-Pos Board B657

Cut7-Driven Microtubule Sliding Reverses Direction Depending on Motor Density

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Cut 7, the only kinesin-5 in S. pombe, is essential for mitosis. We have found that full length Cut7 slides microtubules (MTs) bidirectionally, with MT sliding

velocity and direction dependent on motor crowding and ionic strength but not MT length. By contrast, truncated Cut7 monomers drive only plus end directed MT sliding, indicating that plus end directed strokes are the basal activity of the Cut7 motor head and that directional reversal is an emergent property of interacting head-pairs. We propose a possible mechanism for directional reversal, in which minus ended strokes are inhibited by motor crowding, causing the basal plus end directed activity to dominate.

3930-Pos Board B658

SRC Phosphorylation Regulates the Human Kinesin-5, Eg5, and Disrupts the Binding of Eg5 Inhibitors

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The human kinesin-5 motor, Eg5, is required to establish and maintain the mitotic spindle. We show that Src kinase binds to a unique motif in the microtubule-binding interface of the Eg5 enzymatic head domain and phosphorylates three specific tyrosine residues in endogenous Eg5. These tyrosines are located near the nucleotide pocket and the functionally critical Loop 5 region within the Eg5 head. We have also found that phosphomimetic Eg5 motor proteins have altered motility characteristics relative to wild-type and non-phosphorylatable mutant proteins. Furthermore, cells expressing phosphomimetic Eg5 motors have increased spindle polarity defects. These results implicate Eg5 as a potential direct mitotic target of tyrosine kinases, most likely Src family kinases. Phosphomimetic motors also have greatly reduced affinity for the Eg5 inhibitor S-trityl-L-cysteine (STLC). In cells with high Src activity, including many types of cancers, the same mechanism may provide rapid resistance to therapy with Eg5 inhibitors.

3931-Pos Board B659

Allosteric L5-Directed Inhibitors of Kinesin-5 Can Control Different Biochemical Intermediates

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Kinesin-5 (Eg5) is an essential mitotic motor that couples ATP hydrolysis to different protein-protein association states with microtubules. Human Eg5 is a cancer chemotherapeutic target, having a unique allosteric site covered by loop-5 (L5). This site is capable of binding small-molecule inhibitors with $>$ 100 different chemotypes. The prevailing biochemical model is that all L5-directed drugs inhibit ADP release, but it does not address the 10^7-fold difference in potency or the lack of chemical homology between inhibitor families. An alternative hypothesis is that the inhibitors act on different catalytic intermediates, which gives rise to their disparate potencies. Here we present our linear free energy relationship (LFER) study of Eg5, a method to determine whether an inhibitor can block the catalytic transition-state. Steady-state kinetic parameters for wildtype Eg5 and eight different L5 mutants were determined in the background of three different L5-directed inhibitors and a mock control. The predominant effect of the L5 residue substitution is alteration of substrate binding (K_m), whereas principal outcome of allosteric drug is change in ATP hydrolysis (k_{cat}). Second, our data showed that despite their use of the same binding pocket, one compound can inhibit the transition state and the other two do not. We conclude that the drugs are not synonymous: it is possible for one allosteric modulator to regulate ADP release and another to control transition-state formation. The significance is that this is the first demonstration of allosteric control of more than one catalytic intermediate for any drug target. Furthermore, these results may give insight into the disparity between increased inhibitor potency and success in clinical trials.

This work is funded by the support of the National Institutes of Health (R01 GM097350; S.K.) and the LSU School of Graduate Studies (M.L.).

3932-Pos Board B660

Photo-Reversible Inhibition of Mitotic Kinesin Eg5 by Photochromic STLC Analogues Composed of Azobenzene

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The kinesin Eg5, is a microtubule plus-end directed homotetrameric molecular motor that is essential for the formation of a bipolar spindle during eukaryotic cell division. Eg5 exists only in proliferating cells, and is required for mitosis. Therefore, Eg5 is new target of cancer therapy. It is known that some small molecules specifically inhibit Eg5 activity. S-trityl-L-cysteine (STLC) is one of the potent Eg5 specific inhibitors. In this study, we tried to develop photochromic Eg5 inhibitors that control inhibitory activity of Eg5 by ultraviolet (UV) and visible (VIS) light irradiations reversibly. Azobenzene is one of the typical photochromic molecules, which shows cis, and trans isomerization by UV and VIS light irradiations respectively. In this study, we designed and synthesized photochromic inhibitors utilizing azobenzene. As the trityl group of STLC is a key moiety to exhibit inhibitory activity, we linked the trityl group to N-acetyl cysteine or maleic acid via azobenzene. The synthesized two photochromic inhibitors, TAB-MA and TAB-Ac-Cys inhibited the ATPase activity of Eg5 at the different inhibition constants between cis and trans isomers. Trans-isomers of the inhibitors showed more significant inhibition of ATPase activity than cis-isomers. Moreover, Eg5 driven microtubule gliding was photo controlled by TAB-Ac-Cys. Trans-TAB-Ac-Cys decreased the velocity of microtubule gliding more significantly than cis-TAB-Ac-Cys.

3933-Pos Board B661

Photo-Regulation of Kinesin Eg5 ATPase and Motor Activity using Novel Photochromic Inhibitor Composed of Spiropyran and Cysteine

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The mitotic kinesin Eg5 has an important role in establishing the bipolar spindle which is directly involved in the cell division. Recently, several small molecules have been identified as potent specific inhibitor of Eg5. STLC is one of the inhibitor which does not compete with either ATP or microtubules but slows down ADP release. STLC binds to the pocket on Eg5 composed by a2, a3 and loop L5. STLC binding induces the downward swing of L5 to close the inhibitor binding pocket. Therefore, L5 is one of the key region to stabilize Eg5-STLC complex. Previously, we incorporated photochromic molecules into L5 to photo-control inhibitory activity of STLC. Successfully Eg5 mutant D130C modified with spiropyran derivative showed significant photo-reversible resistance to STLC.

In this study, further application of photochromic molecules to regulate Eg5 activity was performed. We synthesized photochromic STLC analogue, IASP-L-Cys composed of spiropyran and L-cystein to regulate Eg5 ATPase and motor activity reversibly upon ultraviolet and visible lights irradiation. IASP-L-Cys exhibited merocyanine - spiro isomerization upon ultraviolet and visible light irradiations. Zwitterionic merocyanine isomer has a ring-opening structure and differs from the other isomer of spiro that is hydrophobic and ring-closing structure. Therefore, it is expected that photo-isomerization of IASP-L-Cys may alter its inhibitory activity for Eg5 significantly. Microtubule dependent ATPase activity of Eg5 was inhibited by IASP-L-Cys in a concentration dependent manner. And the inhibitory activity of IASP-L-Cys for Eg5 was drastically changed correlating to photo-isomerization. Merocyanine isomer showed much higher inhibition constant than that of spiro isomer (approximately 20 μM and 100 μM, respectively). We also examined the effect of phtoisomerization of IASP-L-Cys for the cell mitosis using HeLa cell.

3934-Pos Board B662

Transducer Residues are Thermodynamically Coupled in the Kinesin-5 Motor Domain

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Motor proteins coordinate the activities of nucleotide hydrolysis and polymer binding to move along cellular tracks. In kinesin and myosin motor domains, the sites responsible for these two activities are located on opposite faces of a core beta-sheet. Three beta-strands and a mobile loop transduce information between the active and polymer-binding sites and, thus, are collectively termed the transducer. Herein we demonstrate residues in the core beta-sheet and the L5 loop are thermodynamically coupled. Two methods were employed in this study. Probabilistic methods to analyze evolutionarily correlations between residues in protein families identified coupling between transducer residues. Second, thermodynamic linkage between M115 in loop-5 and L263 in beta-7 in the human kinesin-5 motor domain was established using double mutant cycle analysis for wildtype, two single mutants, and the corresponding double mutant protein. The resulting changes in free energy were calculated from experimentally measured kinetic parameters and determined to be non-additive. While conformational changes of the beta strands and mobile loop have been observed in structural investigations of kinesins and myosins, these experiments establish energetic and evolutionary coupling of these distinct motifs. We conclude that loop-5